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Percy Edgar Brown
Iowa State College

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Bacteria at Different Depths in Some Typical Iowa Soils.

BY P. E. BROWN

AGRICULTURAL EXPERIMENT STATION
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AGRONOMY SECTION
Soil Bacteriology

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SUMMARY OF CONCLUSIONS

1. In the different soil types, as well as in the same soil under different rotations, the greatest number of organisms occurred at a depth of 4 inches.

2. Bacteria were found in considerable numbers at much lower depths in the loess soil than in the drift soil.

3. There was a more or less gradual decrease in numbers to a depth of 3, 5 and in one case of fifteen feet. No sudden increases were observed even where gains in moisture occurred.

4. The greatest decrease in numbers of organisms occurred within the first 12 inches and in some cases within the first 8 inches.

5. The rotation of crops increased the number of organisms beyond continuous cropping.

6. At 4 inches from the surface, the soil under the three year rotation showed larger numbers than that under any two year rotation, but at 8 inches fewer organisms than the soils under the two year rotation with clover or cowpeas turned under.

7. Rye turned under in the two-year rotation decreased the number of bacteria.

8. Fewer bacteria occurred in the soil under continuous clover than in that under continuous corn, due to the difference in treatment for the crop. Little differences were shown below 12 inches depth.

9. The soil under the four year rotation showed smaller numbers than in any of the plots except those under continuous clover and corn and the two-year rotation with rye turned under, due probably largely to the crop grown.

10. The humus content of the soils in all the plots, except two, and the nitrogen content of all the soils, decreased more or less regularly down to three feet. In the plots under the two-year rotation with clover or cowpeas turned under, there was more humus at eight than at four inches from the surface.

11. While in some cases there seemed to be some relation between numbers and the humus or nitrogen content of the soils, in general the variations observed in these latter were insufficient to account for the differences in numbers. The variations in moisture content of the soils were also insufficient to account for the results.

12. Aeration may be the governing factor, or possibly the effect of toxic substances produced in the growth of plants may be the cause of the variations in the bacterial content of the different plots.

BACTERIA AT DIFFERENT DEPTHS IN SOME TYPICAL IOWA SOILS

BY PERCY EDGAR BROWN.

INTRODUCTION

It is common knowledge that enormous numbers of bacteria are present in cultivated soils and that they influence the rate of availability of plant food. As this rate of availability of plant food largely determines the amount of crop produced, it is a natural inference that there must be some relation between bacterial activities and the crop-producing power of the soil, or its fertility.

Although the value of bacteriological examinations of soils in the past has been limited by the inadequacy of the methods available, recent work has shown some relation between the numbers and physiological activities of organisms in soils, and crop production. Methods now employed give indication of future usefulness and by modifying them it is hoped to make it possible to test soils bacteriologically for their crop-producing power. Even with the very unsatisfactory gelatin and agar media, which were first employed in the work, differences in the bacterial content of soils differently treated were clearly recognized. The zone of most intense bacterial activity was found to be near the surface of the soil; there was a more or less gradual decrease in numbers of organisms with increasing distance from the surface and an entire disappearance at different depths in various soils.

It must be clearly kept in mind that the results obtained in such experiments with one soil are by no means necessarily applicable to any other soil. Differences in the mechanical and chemical composition, not only of the soil but also of the subsoil, and differences in topography, in climatic and weather conditions, etc., all play an important part in governing the numbers and distribution of organisms in different soils. There are two distinct classes of soils in Iowa, the drift areas and the loess soils, and one of the purposes of the work reported in the following pages was to determine the differences in the numbers of bacteria in various soils from these two general types.

The main purpose of this work, however, was to make a careful comparison of the number of organisms at different depths, the moisture conditions, the humus content, and the nitrogen content of eight plots located on the Wisconsin drift area, under different methods of cropping and different rotations. Thus it was sought to determine not only the relative influences of the different methods of cropping on these various factors, but also their influence on the bacterial content of the soils and the depth to which such influence might extend. Furthermore, the relative importance of aeration, moisture, food content, etc., on bacterial action were to be determined as far as possible. It seems probable that the effect of different rotations and methods of cropping on the subsequent yields from the soils is due largely to the effect on the bacterial flora of the soils. In general, therefore, this work may be characterized as an attempt to determine what this effect is. Future study will show how the effect on bacteria leads to subsequent effects on crop yields.

HISTORICAL

Koch in 1881 devised the gelatin plate method for the isolation of pure cultures, and from this work soil bacteriological investigation received the impetus which has carried our knowledge of the subject to its present state of advancement.

The first problems studied were the quantitative estimations of bacteria in different soils and in this connection some work was carried on to determine the numbers of organisms in different layers of soil. Thus in 1882 Proskauer[1] examined the bacterial content of soil at various depths, taking the samples with sterile instruments from the vertical sides of a hole dug for the purpose, and he found that the number or organisms diminished rapidly to a depth of 1½ meters to 2 meters and in some cases was very small at 1 meter depth. Beumer[2], on the other hand, in a soil which was probably contaminated with sewage, found 45,000,000 organisms per cc. at a depth of three meters, 10,000,000 at 4 meters, 8,000,000 at 5 meters, and 5,000,000 at 6 meters depth.

C. Frankel[3] in 1887, made a very careful study of bacteria at different depths and concluded that the number of organisms in a soil increased from the surface down to ¼-½ meter, and then decreased rapidly with increasing depth until at 3 meters they completely disappeared. He found that bacteria went deeper in cultivated than in uncultivated soils, but he was unable to discover any influence of the crop on the number of organisms in the soil. He noted also that in the deeper soil layers there was not a gradual diminution in numbers with increasing depth, but great irregularity with sudden increases or total disappearance of organisms. This was due probably to variations in the physical character of the soil.

Maggiora[1a] found a decrease from over 32,000,000 bacteria

(1) Zeitschr f. Hyg. 11 (1882) p. 22-24.
(2) Dtsche med. Wochenschr, 12 (1886) p. 465.
(3) Zeitschr. f. Hyg. 21 (1887) p. 521; ref. Centbl. f. Bakt. (etc) 3, p. 235.

per gram of soil at the surface to 18,000 organisms at 3 meters depth; and Reimers[2a] concluded that in most cases soils were sterile below 3 to 4 meters depth, although in one instance this was not so at 6 meters, and in several other cases no bacterial development occurred below 2 meters depth. Fulles[1] found that 2 meters marked the limit of bacterial life in most soils and Houston[2] noted a decrease from 1,680,000 bacteria per gram of soil at the surface to 410 organisms at a depth of 6 feet. Caron[3], studying some soils under clover, found a decrease from 6,000,000 organisms per gram of soil at 20 centimeters depth, to 1,500,000 bacteria at 50 centimeters depth. Stoklasa and Ernest[4] examined a soil which decreased from 8,000,000 bacteria at the surface to sterility at 1 meter depth. Chester[5] found that there was an increase in bacteria in soils from the surface to 4 or 6 inches and then a gradual decrease from 1,630,000 (the number present at 4 inches depth) to 4,000 at 24 inches. Eberbach[6] at 1½ to 2 meters depth, obtained an average of 20,000 to 50,000 bacteria per c. c.; while Miquel[7] found 5,400,000 organisms per gram of soil at 2 meters depth in a cemetery soil and Eisenhut[8] 100,000 organisms per cc. of soil at 3 to 4 meters depth.

King and Doryland[9] examined the soil from several plots which were cultivated different numbers of times and found that while increasing numbers of cultivations increased the bacterial content of the soils, the maximum numbers always occurred from the surface to 6 inches and below that a gradual decrease occurred down to 12 inches, at which depth 1,000,000 to 2,000,000 were still present. Stewart and Greaves[10] found nitrification active at 10 feet in a typical Utah soil, and Waite and Squires[11] in a recent experiment with a Nebraska soil showed a gradual decline in numbers from the surface soil to 6 feet; and from that point down to 12 feet, the numbers fluctuated considerably but only one sampling was made and consequently some factor may have accidentally interfered with the results. In comparing the bacteria in soils from under corn and under alfalfa, however, their results showed greater numbers of organisms in the first three feet of corn soil than in the first three feet of alfalfa soil, due prob-

(1a) Glorn, della. R. Accadi di. Medicina 1887 Nr. 3; ref Centbl. f. Bakt. I-1887, p. 677.
(2a) Zeitschr. f. Hyg. 7 (1889) pp. 319-346.
(1) Zeitschr. f. Hyg. 10 (1891) pp. 225-252.
(2) Edinburgh Med. Journ. 1893, p. 112; ref. Centbl. f. Bakt. 14, p. 458.
(3) Landw. Vers. Sta. 45 (1895) p. 404.
(4) Centbl. f. Bakt. II, Abt. 14 (1905) p. 725.
(5) Bul. 65, Del. Expt. Sta., p. 61.
(6) Uber das Verhalten d. Bakt. in Soden. Dorpats. Diss. Med. Dorpats 1890.
(7) Ann. de. MicrMicrogr. 9 (1887) p. 199; ref. Jahresber. d. Garungsorg 8, p. 52.
(8) Uber Terrian Auffullungen usw., Diss. Zurich 1901.
(9) Bul. 161, Kansas Expt. Sta.
(10) Bul. 106, Utah Expt. Sta.
(11) Twenty-fourth Ann. Rept., Nebr. Expt. Sta., p. 160.

ably to the better aeration of the former soil brought about by cultivation.

Löhnis[1] sums up the situation very concisely when he says that the multiplication of organisms is quite different in different soil layers and the number decreases quickly with the depth, air and food being the first considerations. The same author states also that on account of its extensive drying and strong lighting, the surface soil is usually not especially rich in bacteria and the maximum numbers are usually reached at 10 to 20 centimeters depth.

Another fact is noted in the work mentioned. It is not to be expected that bacteria should be evenly distributed through a certain zone, "because the uneven distribution of fermentable material leads to more vigorous growth of bacteria in some parts of the soil than that occurring in other parts." The agreement of parallel determinations is therefore, hardly to be expected. Another reason for this difficulty in securing agreement of duplicate samplings in soils at the same depths may be found in the varying physical character of the soil and the consequent effect on moisture, temperature, aeration, and other conditions.

THE METHODS EMPLOYED

The history of the quantitative examination of soils has been that of a constant effort to devise a medium which will permit of the development of the largest number of organisms. Of course it is manifestly impossible to construct a medium, which, under artificial laboratory conditions will permit of the growth of all soil organisms. Bouillon agar and bouillon gelatin have been used most generally and carefully compared but there are objections to both which render their employment very unsatisfactory. A "synthetic" agar proposed by Lipman and Brown[2] has yielded greater counts than these early media and a "modified synthetic" agar proposed by the same investigators[3] has given still greater numbers.

While recent, still unpublished work of the author has shown that certain media of slightly different composition give still better results, the "modified synthetic" agar was used in this work as the best medium available. This medium is composed thus:—

1000 cc. water
10.00 gms. dextrose
0.50 gm. K_2HPO_4
0.20 gm. $MgSO_4$
0.05 gm. Peptone
20.00 gms. agar

(1) Handb. d. Landw. Bakt. 1910, p. 511
(2) New Jersey Sta. Rpt. 1908, p. 132.
(3) Lipman & Brown, Centbl. f. Bakt. (etc) 2 Abt. 25 (1910) p. 447.

The plates were prepared by the usual dilution method. One hundred gram portions of the fresh samples of soil, obtained as described later, were shaken for five minutes with 200 cc. portions of sterile water. Then the following dilutions were made, sterile pipettes being employed for the transfers:—one cc. of the infusions into 99 cc. sterile water (a); after thoroughly shaking (a), 10 cc. were transferred to 90 cc. of sterile water (b); then 10 cc. of (b) into 90 cc. of sterile water (c); and 10 cc. of (c) into 90 cc. of sterile water (d). One cc. of these dilutions, representing ½, 1-200, 1-2000, 1-20,000, 1-200,000 of a gram of soil, were plated. Duplicate plates were prepared and the results given are the average of the counts obtained. In all cases the counts on the plates which contained one hundred to two hundred colonies were the numbers considered as they were deemed the most accurate. The plates were all incubated for three days at 25 degrees C, at the end of which time the counts were made.

THE METHOD OF SAMPLING

In some preliminary work the samples of soil were drawn from different depths by means of an ordinary soil auger, but it was decided that this method allowed opportunity for contamination of the deeper soil layers from the surface soil even though considerable care was used and Frankel's soil borer was employed for some of the work reported in the following pages. It was found that this method was very slow and laborious and it was impossible to use the borer at all on some soils with very tenacious sub-soils.

Consequently, for most of the work pits were dug and the samples were taken at the proper depths from the sides of these pits. One side was cut down at right angles to the surface soil and by means of a special sterile trowel the samples were taken at the various depths, care being exercised to remove the outer layer of soil before sampling. The soils were then placed in sterile jars and taken to the laboratory where the inoculations were performed at once, uniformity of samples being carefully obtained and all precautions observed in making the infusions and dilutions. In all cases samples were taken every 4 inches to a depth of 2 feet and then every 6 inches, in Series I, to 3 feet, in Series II, to 15 feet, and in Series III and the subsequent Series, to a depth of 5 feet.

SERIES I.

In this series, samples of soil were drawn as has been described, from eight plots which are being used for experimental purposes by this department. These plots are located on the Wisconsin drift area and the soil is classed as Marshall loam by the Bureau of Soils. They are under the careful management employed in all plot experiments, and have been in the present experiment for four years. The special treatment of the plots was as follows:

Plot No.	Treatment.
601	Continuous corn.
602	2 year rotation, corn and oats.
603	3 year rotation, corn, oats and clover.
607	2 year rotation, corn and oats, clover turned under.
609	2 year rotation, corn and oats, cowpeas turned under.
901	2 year rotation, corn and oats, rye turned under.
903	Continuous clover.
904	4 year rotation, corn, corn, oats and clover.

During the season of the experiment, the first six of these plots were in corn while plots 903 and 904 were in clover, so the results from these latter soils are not strictly comparable with the others, as the difference in treatment for corn and for clover might be expected to alter bacterial relations considerably. Four samplings in all were made, the first, September 16; the second, September 23; the third, September 30, and the fourth, October 7. The samples were drawn as has been described and the entire sixty-four were plated the same day. Duplicate plates were prepared and the results of the counts obtained after three days' incubation may be found in table I. Moisture determinations were made in the samples and the results of these are given in table II. Samples taken similarly to these used for bacteriological examination were dried, ground and analyzed for total nitrogen by the regular Kjeldahl method, and for humus by the modification of the official method which is described in Bulletin 124 of the Iowa agricultural experiment station. The results of the humus determinations are given in table III, and of the nitrogen determinations in table IV.

Turning now to table I for the results of the quantitative determinations, we note that in every case there was a large decrease in numbers of organisms from 4 inches depth to 3 feet, only about 30,000 per gram of air-dry soil being present at the latter depth as against two to four millions at 4 inches. Furthermore, the decrease was continuous. There were no sudden increases in numbers of organisms at lower depths such as have been noted by other investigators. While some differences between the different samplings from the same plots were apparent, the results on the whole agreed very well and in only a few cases were different relations between the results from the various pots, at different samplings, brought out in the table and in these few cases the differences were small. Another point appears quite clearly in looking over the results as a whole, and that is that in every case the greatest numbers were found in the first samples, taken at

TABLE I. SERIES I.

Plot No.	Lab. No.	Depth of Sampling	Bacteria Per Gram of Air-dry Soil I.	II.	III.	IV.	Average
601	A	4 in.	2,033,000	1,627,000	1,793,000	1,555,000	1,752,000
	B	8 in.	1,437,000	1,211,000	1,241,000	1,104,000	1,248,250
	C	12 in.	541,000	567,000	559,000	525,000	546,000
	D	16 in.	287,000	292,000	312,000	302,000	298,250
	E	20 in.	147,000	154,000	159,000	154,000	153,500
	F	24 in.	92,300	96,500	95,100	91,500	93,850
	G	30 in.	49,900	46,300	50,900	46,900	48,500
	H	36 in.	32,900	30,000	33,100	30,400	31,600
602	A	4 in.	3,102,000	2,870,000	2,917,000	2,947,000	2,959,000
	B	8 in.	2,238,000	2,177,000	2,105,000	2,258,000	2,194,500
	C	12 in.	498,000	531,000	531,000	528,000	522,000
	D	16 in.	255,000	328,000	316,000	314,000	304,250
	E	20 in.	182,000	192,000	188,000	177,000	184,750
	F	24 in.	89,200	93,300	91,600	88,300	90,600
	G	30 in.	53,300	54,900	53,100	51,800	54,275
	H	36 in.	31,700	35,700	34,200	31,300	33,225
604	A	4 in.	4,606,000	3,908,000	4,210,000	3,932,000	4,164,000
	B	8 in.	3,132,000	2,834,000	2,976,000	2,793,000	2,943,750
	C	12 in.	1,016,000	882,000	901,000	831,000	907,500
	D	16 in.	320,000	309,000	311,000	320,000	315,000
	E	20 in.	155,000	163,000	156,000	149,000	155,750
	F	24 in.	89,400	96,100	92,900	88,900	91,825
	G	30 in.	51,900	55,800	55,000	52,400	53,775
	H	36 in.	35,100	36,600	34,900	32,600	34,800
607	A	4 in.	4,300,000	4,197,000	4,025,000	4,071,000	4,148,250
	B	8 in.	3,726,000	3,726,000	3,400,000	3,512,000	3,591,000
	C	12 in.	1,192,000	1,190,000	1,162,000	1,127,000	1,167,750
	D	16 in.	339,000	351,000	361,000	342,000	348,250
	E	20 in.	239,000	213,000	226,000	214,000	223,000
	F	24 in.	114,000	111,000	109,000	101,000	108,750
	G	30 in.	58,600	**62,000**	61,100	58,800	60,125
	H	**36 in.**	36,400	**37,600**	38,000	38,500	37,625

TABLE I. SERIES I. (Continued.)

Plot No.	Lab. No.	Depth of Sampling	Bacteria Per Gram of Air-dry Soil I.	II.	III.	IV.	Average
609	A	4 in.	3,880,000	3,928,000	4,000,000	3,809,000	3,904,250
	B	8 in.	2,874,000	2,922,000	3,017,000	2,992,000	2,951,250
	C	12 in.	651,000	646,000	680,000	635,000	653,000
	D	16 in.	281,000	305,000	300,000	329,000	303,750
	E	20 in.	189,000	205,000	196,000	205,000	198,750
	F	24 in.	91,100	92,900	91,100	89,800	91,225
	G	30 in.	56,300	54,500	53,100	51,200	53,775
	H	36 in.	34,700	35,300	34,400	32,900	34,325
901	A	4 in.	2,816,000	2,666,000	2,635,000	2,605,000	2,680,500
	B	8 in.	2,000,000	1,977,000	1,906,000	1,931,000	1,953,500
	C	12 in.	464,000	487,000	496,000	485,000	483,000
	D	16 in.	215,000	263,000	268,000	240,000	246,500
	E	20 in.	149,000	167,000	173,000	148,000	159,250
	F	24 in.	84,100	88,800	91,100	86,700	87,675
	G	30 in.	49,200	52,400	51,500	49,700	50,700
	H	36 in.	29,500	33,300	33,700	31,600	32,025
903	A	4 in.	1,634,000	1,227,000	1,195,000	1,287,000	1,335,750
	B	8 in.	1,700,000	662,000	723,000	822,000	844,250
	C	12 in.	445,000	363,000	372,000	393,000	393,250
	D	16 in.	237,000	246,000	250,000	260,000	248,250
	E	20 in.	173,000	187,000	169,000	176,000	176,250
	F	24 in.	86,400	88,600	89,700	88,300	88,250
	G	30 in.	48,300	46,900	49,300	46,800	47,825
	H	36 in.	31,100	32,100	32,400	30,900	31,625
904	A	4 in.	2,994,000	2,971,000	2,941,000	2,742,000	2,912,000
	B	8 in.	2,130,000	1,977,000	2,070,000	1,931,000	2,027,000
	C	12 in.	553,000	569,000	575,000	545,000	560,500
	D	16 in.	305,000	329,000	323,000	307,000	316,000
	E	20 in.	223,000	271,000	280,000	250,000	256,000
	F	24 in.	86,400	89,300	90,700	90,500	89,225
	G	30 in.	47,300	49,600	49,100	50,100	49,025
	H	36 in.	30,700	33,500	32,600	33,100	32,475

TABLE II. SERIES I.

Plot No.	Lab. No.	Depth of Sampling	MOISTURE I.	II.	IN III.	PERCENT IV.
601	A	4 in.	16.40	11.50	13.00	10.00
	B	8 in.	16.50	12.50	13.00	9.50
	C	12 in.	15.00	12.50	13.50	9.50
	D	16 in.	9.50	10.50	13.50	10.00
	E	20 in.	9.00	10.50	12.00	9.50
	F	24 in.	9.00	10.50	10.50	8.00
	G	30 in.	9.50	8.00	10.50	8.00
	H	36 in.	9.00	8.00	9.50	8.00
602	A	4 in.	16.20	15.00	15.00	14.50
	B	8 in.	16.00	15.50	14.50	15.00
	C	12 in.	11.60	13.50	13.50	14.00
	D	16 in.	9.50	13.50	11.50	13.50
	E	20 in.	10.00	11.50	11.00	10.00
	F	24 in.	10.40	11.50	10.50	9.50
	G	30 in.	10.00	11.50	10.50	9.00
	H	36 in.	8.40	10.50	9.50	9.50
604	A	4 in.	17.50	12.50	14.50	11.00
	B	8 in.	17.00	12.50	14.00	10.50
	C	12 in.	17.00	12.50	13.50	11.00
	D	16 in.	15.00	12.00	11.50	9.50
	E	20 in.	12.50	12.00	10.50	9.00
	F	24 in.	10.60	11.00	10.50	8.50
	G	30 in.	8.00	10.50	10.00	8.50
	H	36 in.	9.00	10.50	9.50	8.00
607	A	4 in.	21.00	19.00	20.00	18.50
	B	8 in.	19.50	19.50	20.00	18.00
	C	12 in.	19.50	19.00	19.50	17.50
	D	16 in.	17.50	18.00	17.00	17.00
	E	20 in.	18.00	15.50	17.00	16.00
	F	24 in.	16.50	15.50	16.00	15.50
	G	30 in.	16.50	15.50	16.00	15.00
	H	36 in.	16.50	15.00	16.00	15.00
609	A	4 in.	16.50	16.50	17.50	16.00
	B	8 in.	16.50	16.50	16.50	15.50
	C	12 in.	14.00	15.50	16.50	15.00
	D	16 in.	9.00	11.00	12.00	15.00
	E	20 in.	9.00	10.50	10.50	12.50
	F	24 in.	10.00	10.50	10.00	11.00
	G	30 in.	12.00	10.50	10.50	9.50
	H	36 in.	12.50	10.50	10.00	9.00
901	A	4 in.	14.80	13.00	15.00	12.50
	B	8 in.	14.00	13.00	14.00	13.00
	C	12 in.	9.50	10.50	13.00	13.00
	D	16 in.	8.00	10.50	10.50	12.00
	E	20 in.	7.50	10.50	10.50	11.00
	F	24 in.	7.60	10.00	10.00	9.50
	G	30 in.	7.00	10.00	10.00	10.00
	H	36 in.	6.50	10.00	10.00	9.00
903	A	4 in.	18.00	12.00	13.00	13.00
	B	8 in.	18.00	12.50	11.50	12.50
	C	12 in.	16.50	12.00	11.50	11.00
	D	16 in.	9.00	11.50	10.50	10.00
	E	20 in.	8.00	10.50	10.50	9.50
	F	24 in.	7.50	8.00	10.00	9.50

TABLE II. SERIES I. (Continued.)

Plot No.	Lab. No.	Depth of Sampling	MOISTURE I.	IN II.	III.	PERCENT IV.
903	G	30 in.	9.00	8.00	10.00	9.50
	H	36 in.	7.50	8.00	10.00	9.50
904	A	4 in.	14.50	12.50	15.00	12.50
	B	8 in.	15.50	13.00	15.00	13.00
	C	12 in.	14.00	15.00	14.50	12.00
	D	16 in.	13.50	15.00	13.50	11.00
	E	20 in.	10.50	14.50	11.00	10.50
	F	24 in.	7.50	10.50	10.50	9.00
	G	30 in.	8.00	10.50	10.50	9.00
	H	36 in.	9.00	10.50	10.50	9.50

TABLE III. SERIES I.

Plot No.	Lab. No.	Depth of Sampling	Humus in Percent
601	A	4 in.	3.55
	B	8 in.	3.33
	C	12 in.	3.21
	D	16 in	2.92
	E	20 in.	2.64
	F	24 in.	2.38
	G	30 in.	2.12
	H	36 in.	1.93
602	A	4 in.	3.98
	B	8 in.	3.60
	C	12 in.	3.30
	D	16 in.	3.18
	E	20 in.	2.98
	F	24 in.	2.66
	G	30 in.	2.11
	H	36 in.	1.29
604	A	4 in.	3.20
	B	8 in.	3.29
	C	12 in.	3.00
	D	16 in.	2.53
	E	20 in.	2.32
	F	24 in.	1.92
	G	30 in.	1.67
	H	36 in.	1.12
607	A	4 in.	3.29
	B	8 in.	3.70
	C	12 in.	2.92
	D	16 in.	2.54
	E	20 in.	2.46
	F	24 in.	2.10
	G	30 in.	1.50
	H	36 in.	0.85
609	A	4 in.	3.46
	B	8 in.	3.63
	C	12 in.	3.12
	D	16 in.	2.93
	E	20 in.	2.56

TABLE III. SERIES I. (Continued.)

Plot No.	Lab. No.	Depth of Sampling	Humus in Percent
609	F	24 in.	2.18
	G	30 in.	1.66
	H	36 in.	1.32
901	A	4 in.	3.00
	B	8 in.	2.87
	C	12 in.	2.87
	D	16 in.	2.34
	E	20 in.	2.10
	F	24 in.	2.04
	G	30 in.	1.93
	H	36 in.	1.51
903	A	4 in.	3.74
	B	8 in.	3.47
	C	12 in.	3.17
	D	16 in.	2.50
	E	20 in.	2.27
	F	24 in.	2.25
	G	30 in.	1.77
	H	36 in.	1.49
904	A	4 in.	3.14
	B	8 in.	2.94
	C	12 in.	2.82
	D	16 in.	2.35
	E	20 in.	1.92
	F	24 in.	1.61
	G	30 in.	1.39
	H	36 in.	0.92

TABLE IV. SERIES I.

Plot No.	Lab. No.	Depth of Sampling	Nitrogen in Percent
601	A	4 in.	0.2465
	B	8 in.	0.2335
	C	12 in.	0.2305
	D	16 in.	0.1531
	E	20 in.	0.1012
	F	24 in.	0.0882
	G	30 in.	0.0701
	H	36 in.	0.0337
602	A	4 in.	0.2621
	B	8 in.	0.2336
	C	12 in.	0.1765
	D	16 in.	0.1583
	E	20 in.	0.1220
	F	24 in.	0.1012
	G	30 in.	0.0882
	H	36 in.	0.0441
604	A	4 in.	0.2672
	B	8 in.	0.2284
	C	12 in.	0.2050
	D	16 in.	0.1583

TABLE IV. SERIES I. (Continued.)

Plot No.	Lab. No.	Depth of Sampling	Nitrogen in Percent
604	E	20 in.	0.1271
	F	24 in.	0.0960
	G	30 in.	0.0675
	H	36 in.	0.0441
607	A	4 in.	0.2518
	B	8 in.	0.2362
	C	12 in.	0.1739
	D	16 in.	0.1479
	E	20 in.	0.1246
	F	24 in.	0.1038
	G	30 in.	0.0727
	H	36 in.	0.0441
609	A	4 in.	0.2465
	B	8 in.	0.2310
	C	12 in.	0.1635
	D	16 in.	0.1271
	E	20 in.	0.1090
	F	24 in.	0.0856
	G	30 in.	0.0701
	H	36 in.	0.0493
901	A	4 in.	0.2310
	B	8 in.	0.2050
	C	12 in.	0.1790
	D	16 in.	0.1245
	E	20 in.	0.1064
	F	24 in.	0.0779
	G	30 in.	0.0545
	H	36 in.	0.0259
903	A	4 in.	0.2232
	B	8 in.	0.2102
	C	12 in.	0.1661
	D	16 in.	0.1375
	E	20 in.	0.1168
	F	24 in.	0.0986
	G	30 in.	0.0831
	H	36 in.	0.0493
904	A	4 in.	0.2050
	B	8 in.	0.1791
	C	12 in.	0.1427
	D	16 in.	0.1064
	E	20 in.	0.0856
	F	24 in.	0.0727
	G	30 in.	0.0441
	H	36 in.	0.0233

4 inches from the surface. Thus former observations are confirmed and at least in this particular type of soil there can be no doubt but that the main zone of bacterial activity lies about 4 inches below the surface, a large decrease occurring before the eighth inch is reached.

We find also that while there was this decrease between the fourth and eighth inches from the surface, a much greater decrease occurred in passing from the eighth to the twelfth inch. Below that the decreases were not so great.

The results of the four samplings of plot 601 which was in continuous corn are presented graphically in plate I. Some irregularities are noticed in the counts obtained in this plot down to 12 inches but turning to table II and also to the curves for moisture content in plate I we find that the moisture in the soil at the first three depths was somewhat greater at the first sampling than at the others. Below the twelfth inch depth the moisture conditions were more uniform and the counts obtained at the different samplings were very good duplicates. Considering therefore the averages of the results obtained at the four samplings there was a more or less gradual decrease in numbers down to 3 feet in this plot, the greatest decrease occurring between the eighth and the twelfth inches. This decrease occurred notwithstanding practically uniform moisture conditions at all the samplings except the first.

Furthermore, from 20 inches down to 3 feet, there was little variation in moisture content of the soil but there was a rather rapid decrease in numbers of organisms. The results of the humus determinations in this plot show that there was a gradual decrease in humus content in the soil from 4 inches down to 3 feet the curve for this being almost a straight line. There is some correspondence here between the decrease in humus and in numbers but the largest decrease in numbers which was observed between the eighth and twelfth inches was evidently not due to a large decrease in humus, for the difference in humus at these two depths was very slight. The results of the nitrogen determinations also show some relation to numbers, a gradual decrease in nitrogen content being observed, corresponding to that in organisms. But while the greatest decrease in nitrogen occurred between the twelfth and sixteenth inches, the largest drop in numbers occurred between the eighth and twelfth inches, so that the nitrogen content of the soil was evidently not the governing factor for bacterial growth.

It may be noted here, therefore, that under practically uniform moisture conditions, below 12 inches the numbers of organisms followed very closely the diminishing humus and nitrogen content of the soil. Nearer the surface, however, some other factor evidently was of more importance and overcame the effects not only of differences in food but also of differences in moisture.

Turning to plate II we find that plot 602, which was under a two-year rotation of corn and oats, showed a decreasing bacterial content from 4 inches down to 3 feet. The greatest decrease between any two layers sampled occurred between the eighth and twelfth inches when a drop from over 2,000,-

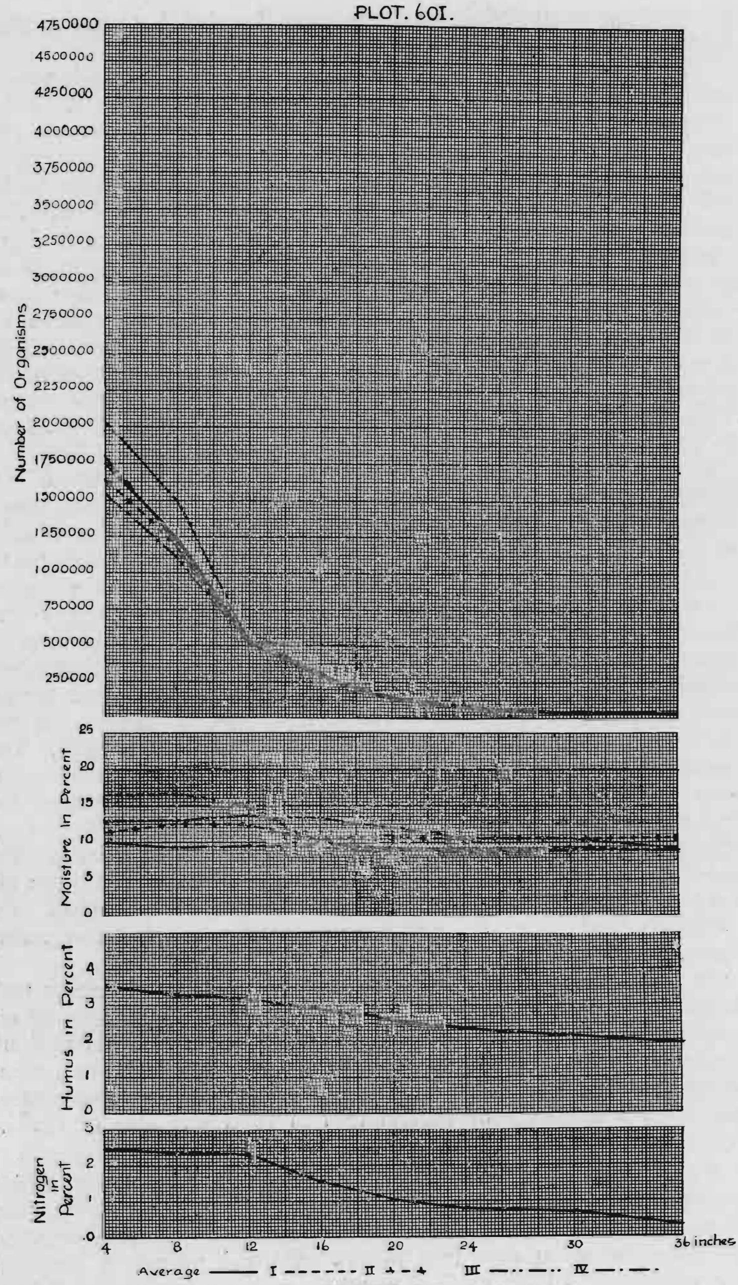
PLOT. 601.
Number of Organisms
4750000
4500000
4250000
4000000
3750000
3500000
3250000
3000000
2750000
2500000
2250000
2000000
1750000
1500000
1250000
1000000
750000
500000
250000
Moisture in Percent
25
20
15
10
5
0
Humus in Percent
4
3
2
1
0
Nitrogen in Percent
3
2
1
.0
4
8
12
16
20
24
30
36 inches
Average I II III IV

PLATE I.

000 bacteria to 500,000 per gram of soil was observed. Below this depth a more or less gradual decrease occurred down to 3 feet. The results of the four samplings of this plot as may be seen from the curves were quite uniform. At the first sampling the counts obtained at 12 and 16 inches were lower than those from the same layers at the three later samplings, but we find here again that a low moisture content in those two samples at the first date might account for the divergence in results. In all the other cases when the moisture conditions were more or less uniform the results of the four samplings were in good agreement.

The humus and nitrogen determinations in the soils from this plot show again a more or less rapid decline corresponding to the decrease in numbers. The curves for the humus and nitrogen content of this plot at the different depths are almost straight lines.

Again it appears that down to 12 inches or possibly somewhat below that, the numbers of organisms decrease much more rapidly than the humus or nitrogen content while below that depth, under practically constant moisture conditions, the numbers decrease with the nitrogen and humus content.

Plate III presents graphically the results obtained from the soil from plot 604 which was under a three-year rotation of corn, oats and clover. At the first three depths somewhat greater counts were obtained at the first sampling than at the three later dates and the moisture table shows that the moisture content of the first samples at the first three depths was greater than that of the later samples at those depths. At the lower depths the moisture conditions were much more uniform and the counts obtained agreed very satisfactorily. The greatest drop in numbers between any two samples occurred here again between the eighth and twelfth inches and this notwithstanding quite uniform moisture conditions.

The humus content of the soil from this plot decreased gradually from eight inches down to three feet, the amount present at 8 inches being practically the same as that present at 4 inches. The nitrogen content also decreased gradually with increasing distance from the surface and so again the large decrease in numbers from 4 inches to 16 inches cannot be attributed entirely to decreasing food supply as this was very slightly reduced but must be due to some other factor.

In plate IV may be found the curves showing the results from plot 607 which was under a two-year rotation of corn and oats with clover plowed under for a green manure. The results were very uniform no large differences being apparent between the results from any depth at different samplings. The moisture conditions at the four samplings were very uni-

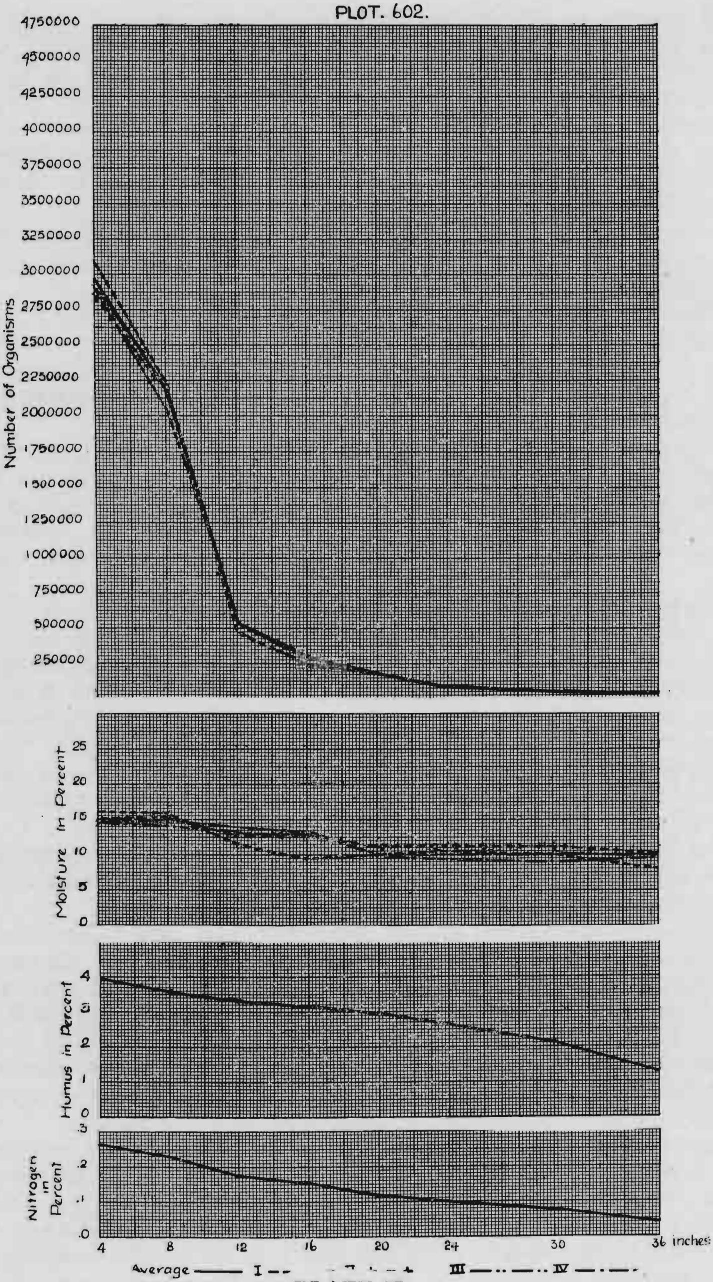
PLOT. 602.
Number of Organisms
Moisture in Percent
Humus in Percent
Nitrogen in Percent
inches
Average
I
III
IV

PLATE II.

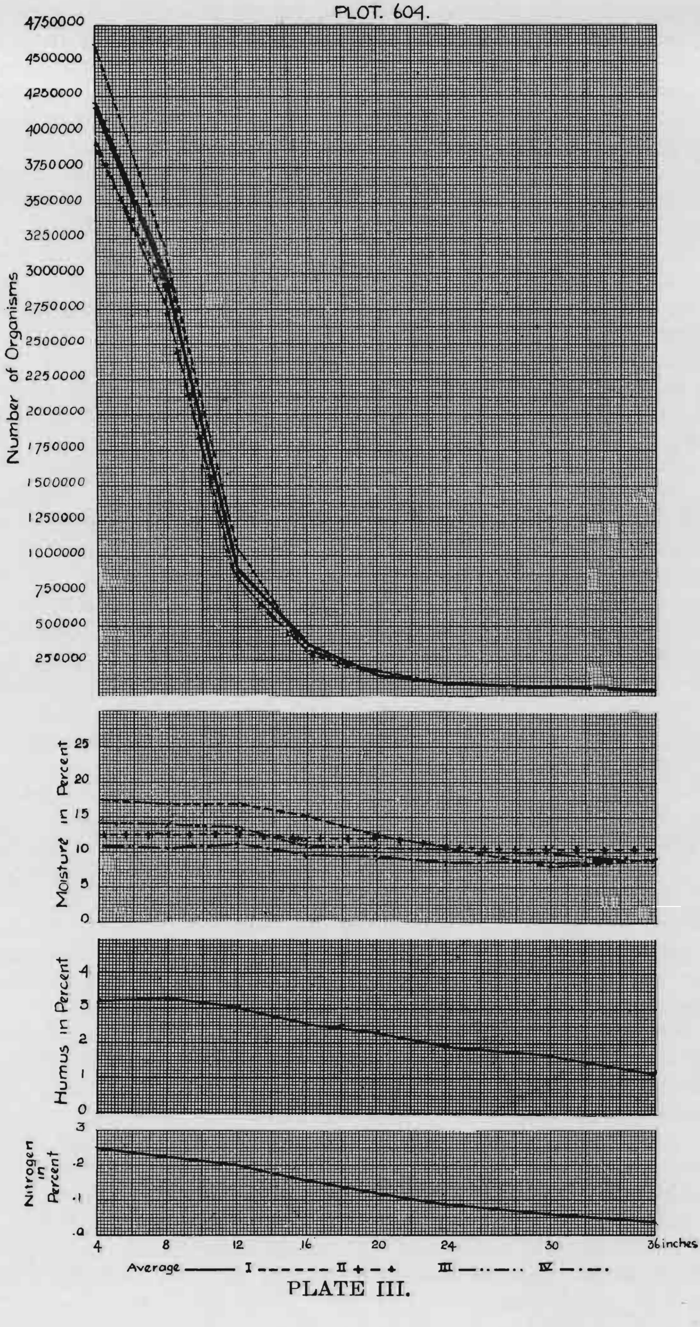
PLOT. 604.
4750000
4500000
4250000
4000000
3750000
3500000
3250000
3000000
2750000
2500000
2250000
2000000
1750000
1500000
1250000
1000000
750000
500000
250000
Number of Organisms
25
20
15
10
5
0
Moisture in Percent
4
3
2
1
0
Humus in Percent
.3
.2
.1
.0
Nitrogen in Percent
4
8
12
16
20
24
30
36 inches
Average
I
II
III
IV

PLATE III.

form also for the various layers as is evidenced by the curves and the better agreement of the results was evidently largely due to this fact. The greatest decrease between any two depths occurred here again between the eighth and twelfth inches and the greatest drop in the humus and nitrogen content of the soils appeared also at these depths but the differences in these cases were not nearly so pronounced, the nitrogen and humus content decreasing more gradually down to three feet. One exception to this decrease in the case of the humus content must be noted. At 8 inches we find a greater amount of humus present in the soil than at 4 inches. The clover turned under in this plot evidently increased the humus content of the soil at the lower depth.

Turning now to plate V which presents the results for plot 609 we find them quite uniform also; one exception occurring at 16 inches at the first sampling when a low count was obtained and a low moisture content was also observed. At the other dates the numbers obtained were not variable to any extent and the slight differences in moisture content seemed not to cause any noticeable effect on the number of bacteria. The nitrogen and humus content of the soils show here a gradual decrease, with the exception that the soil at 8 inches from the surface contained more humus than that at 4 inches from the surface. Here again the green manure crop showed its effect on the humus content of the soil, not only increasing the total humus in the soil but also causing larger amounts to be present at lower depths. The decrease in nitrogen and humus content of the soils was very much more gradual than the decrease in bacteria and so some other factor evidently influenced the number of organisms present near the surface of the soil much more than food supply.

Plate VI gives the results of the examination of plot 901 and it will be seen that the results of the four samplings were very uniform. The moisture conditions were likewise quite uniform except at the first sampling. At that date at all the depths except the first two, the moisture content was somewhat lower than that of the samples at those depths at later samplings. Although the curves do not show it clearly table I shows that the numbers at the first sampling at these depths were somewhat smaller than those obtained at later samplings at the same depths. The humus and nitrogen content of the samples from this plot decreased gradually with increasing distance from the surface. In both cases the greatest drop occurred between the twelfth and the sixteenth inches and beyond that there was a very gradual decrease.

Plots 903 and 904, as has already been mentioned, were in clover during the season of the experiment while the other plots were in corn so that the results are not strictly com-

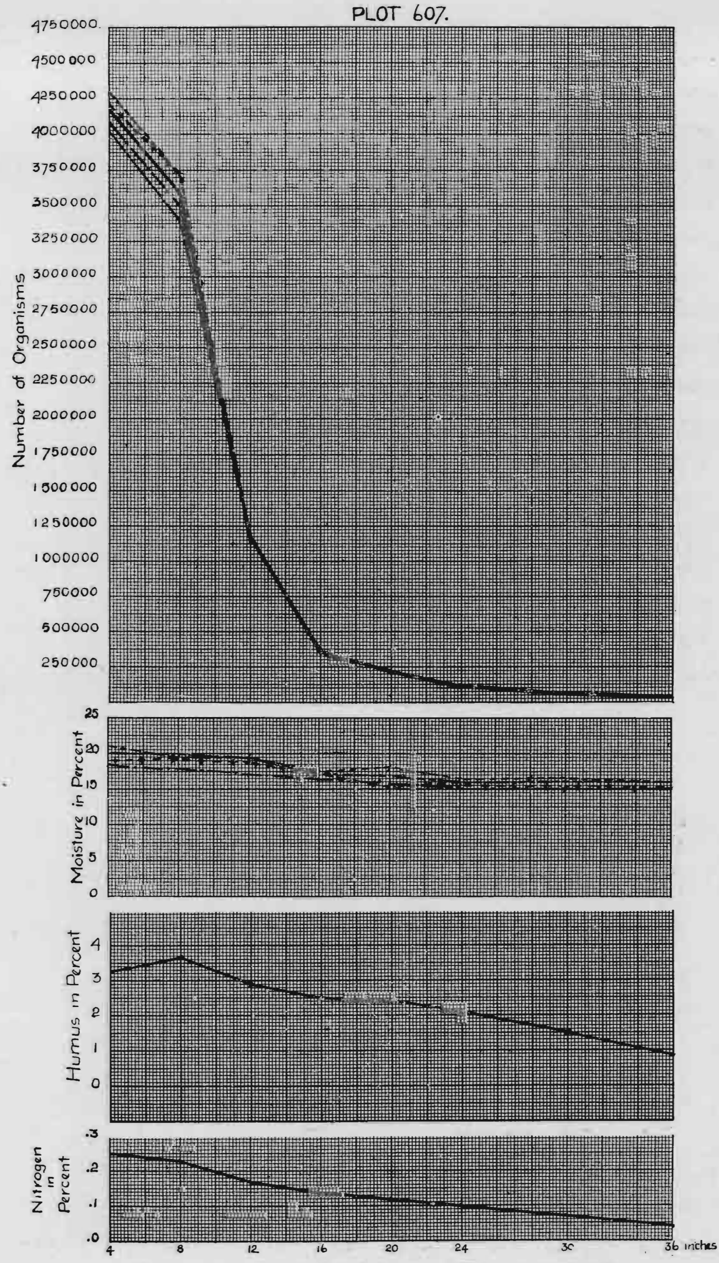
PLOT 607.
4750000
4500000
4250000
4000000
3750000
3500000
3250000
3000000
2750000
2500000
2250000
2000000
1750000
1500000
1250000
1000000
750000
500000
250000
Number of Organisms
25
20
15
10
5
0
Moisture in Percent
4
3
2
1
0
Humus in Percent
.3
.2
.1
.0
Nitrogen in Percent
4
8
12
16
20
24
30
36 inches
Average
I
II
III
IV

PLATE IV.

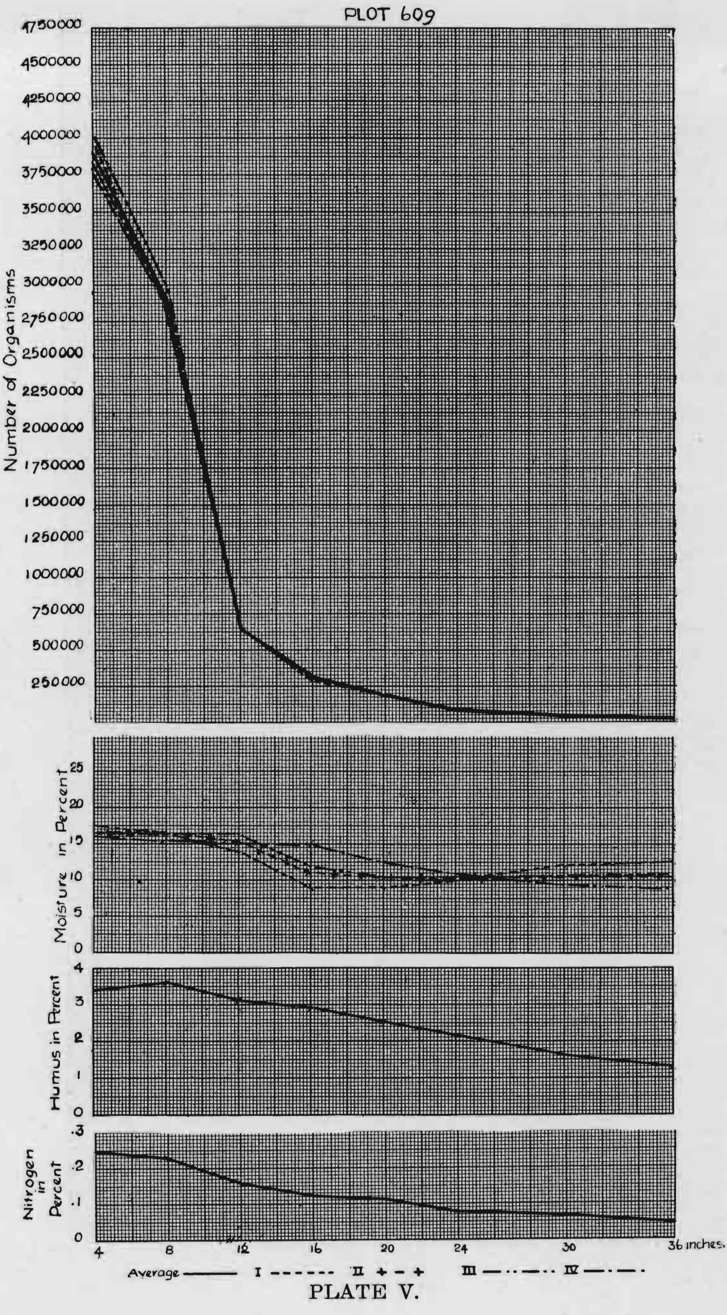
PLOT 609
Number of Organisms
4750000
4500000
4250000
4000000
3750000
3500000
3250000
3000000
2750000
2500000
2250000
2000000
1750000
1500000
1250000
1000000
750000
500000
250000
Moisture in Percent
25
20
15
10
5
0
Humus in Percent
4
3
2
1
0
Nitrogen in Percent
.3
.2
.1
0
4
8
12
16
20
24
30
36 inches.
Average
I
II
III
IV

PLATE V.

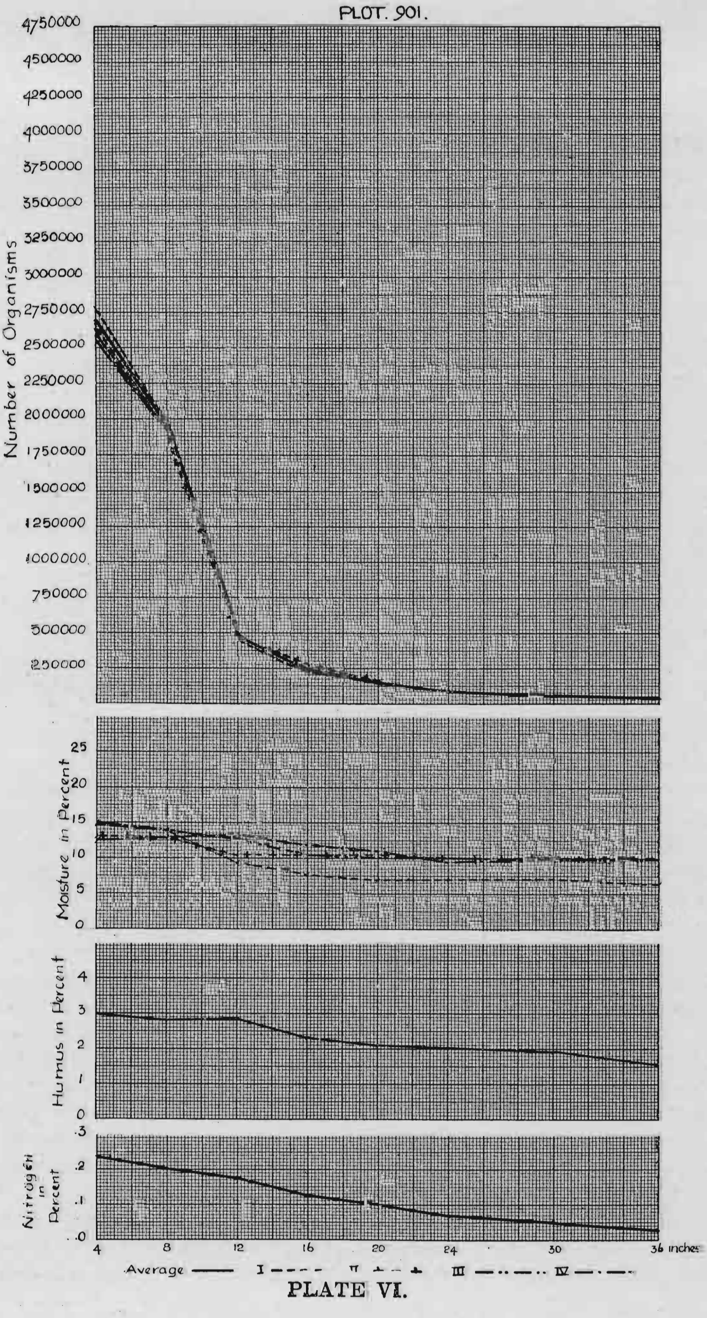
PLOT. 901.
Number of Organisms
4750000
4500000
4250000
4000000
3750000
3500000
3250000
3000000
2750000
2500000
2250000
2000000
1750000
1500000
1250000
1000000
750000
500000
250000
Moisture in Percent
25
20
15
10
5
0
Humus in Percent
4
3
2
1
0
Nitrogen in Percent
.3
.2
.1
.0
4
8
12
16
20
24
30
36 inches
Average
I
II
III
IV

PLATE VI.

parable. The figures obtained are interesting, however, in showing the effect of a different crop on the bacteria in the soil and also in showing the relative effects of present and previous cropping.

Plates VII and VIII show the results obtained in the examination of the soils from these plots. The numbers of organisms found in the soil from plot 903 at the first sampling were much greater at the fourth and eighth inch depths than those obtained at the later samplings. The moisture content of the soil at these depths at this sampling was greater than that of the samples at the same depths at the other samplings and this difference was probably largely responsible for the variations in the results. The moisture conditions at the other depths for all the samplings were quite uniform and the counts were likewise much less variable and quite satisfactory.

The greatest decrease in numbers occurred here again between the eighth and twelfth inches and this drop occurred notwithstanding very slightly decreased moisture content. The humus content in plot 903 proved somewhat variable but still gradually decreasing and likewise the nitrogen content; both decreasing much more gradually than the numbers.

In plot 904 the numbers obtained at the different depths at different samplings were very uniform and again the greatest decrease between any two depths occurred between the eighth and the twelfth inches. The moisture conditions were likewise not variable to any great extent and the humus and nitrogen curves are practically straight lines.

Plate IX gives a summarized picture of the average results from the eight plots.

Plot 602, under the two-year rotation, showed much greater numbers of organisms present at both the fourth and eighth inch depths than plot 601 under continuous corn, while below 8 inches the numbers were practically the same. The moisture content and the humus content of the samples from these two depths in plot 602 were greater than of the samples at similar depths in plot 601, so that either or both of these factors may have had some influence. The difference in moisture, humus, and nitrogen content of the soils from these two plots was, however, too slight to explain entirely the diminished numbers of organisms in the soil. Evidently here the effect of the two-year rotation in increasing the number of organisms in the soil over that in the continuous corn soil is clearly pronounced and this effect seems much greater than could be accounted for by the comparatively small differences in moisture, humus, or nitrogen content.

In plot 604 under a three-year rotation, much greater numbers of organisms were obtained at the first, second, and third depths than in the other two plots while below the third depth,

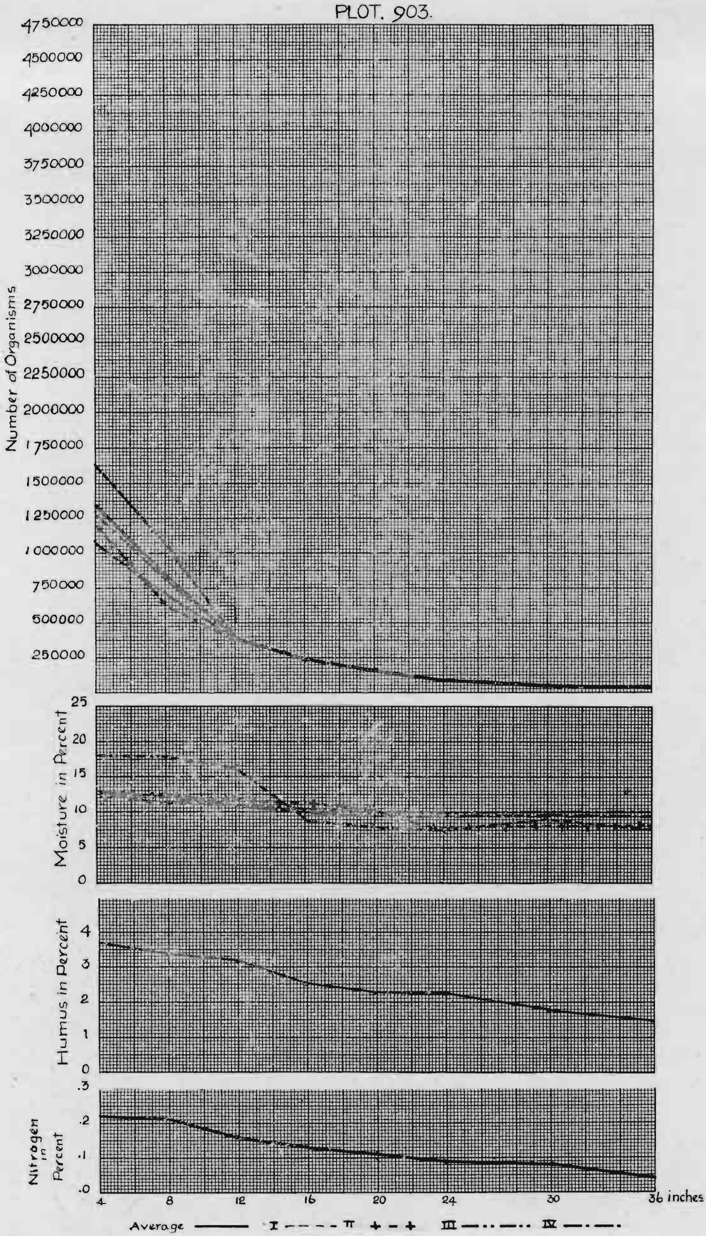
PLOT. 903.
Number of Organisms
4750000
4500000
4250000
4000000
3750000
3500000
3250000
3000000
2750000
2500000
2250000
2000000
1750000
1500000
1250000
1000000
750000
500000
250000
Moisture in Percent
25
20
15
10
5
0
Humus in Percent
4
3
2
1
0
Nitrogen in Percent
.3
.2
.1
.0
4
8
12
16
20
24
30
36 inches
Average
I
II
III
IV

PLATE VII.

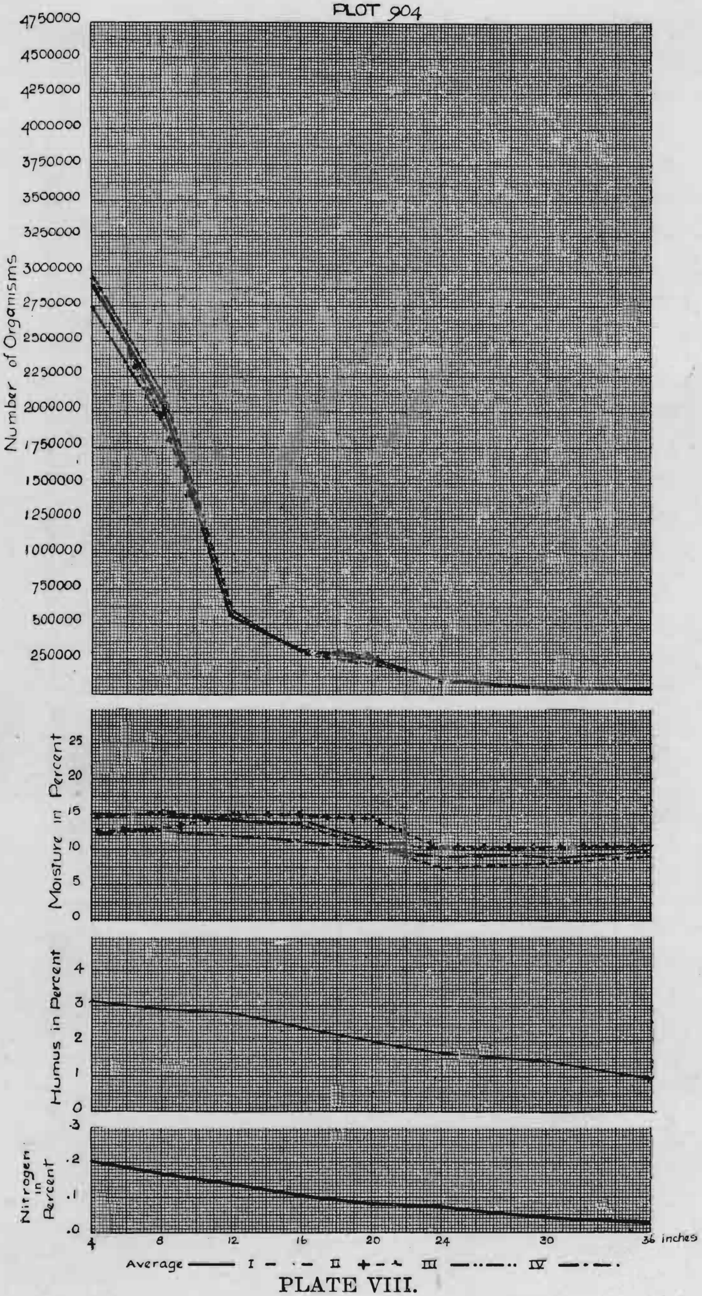
PLOT 904
4750000
4500000
4250000
4000000
3750000
3500000
3250000
3000000
2750000
2500000
2250000
2000000
1750000
1500000
1250000
1000000
750000
500000
250000
Number of Organisms
Moisture in Percent
25
20
15
10
5
0
Humus in Percent
4
3
2
1
0
Nitrogen in Percent
.3
.2
.1
.0
4
8
12
16
20
24
30
36 inches
Average
I
II
III
IV

PLATE VIII.

the differences were very slight. The moisture, humus and nitrogen contents of the samples were practically the same as those of plots 601 and 602 and consequently none of these factors can be held accountable for the results but a further cause must be sought. The three-year rotation here showed greater numbers of organisms than either the continuous corn plot or the two-year rotation plot.

Considering the average results from plot 607 which was under a two-year rotation with clover turned under for a green manure, the same number of organisms at 4 inches was found as in plot 604 and more than in plot 602. At 8 inches however, a considerably larger number of organisms was present in plot 607 than in the others and the same was true at the twelve inch depth.

Below that point the differences from the other plots were somewhat smaller but at every depth down to 3 feet greater numbers were present in plot 607. The moisture conditions in this plot were uniformly higher than in the other plots, the greatest differences being apparent at the lower depths. The percentage of humus in plot 607 was greater at the fourth inch than in plot 604 and still greater at the eighth inch but the differences were not very large and the only noticeable fact was that the humus content of the soil at 8 inches from the surface was greater than that at four inches, 3.70% against 3.29% and the effect of turning under a crop of green manure on the humus content of the soil at deeper layers was evidenced. The nitrogen content of the soils from this plot was not very different from that of the other plots and consequently none of these factors examined is sufficient to explain the results. It is evident however, that turning under a crop of green manure such as clover, in a two-year rotation increased the bacterial content of the soil beyond that of an ordinary two-year rotation. While at 4 inches from the surface no gain over the bacterial content of the soil under the three-year rotation was noticeable, at the eighth and twelfth inches greater numbers of organisms were found to be present in this plot than in the plot under the regular three-year rotation. In other words, turning under a crop of clover increased the total number of organisms in the soil more than cropping to clover in the regular way. This increase was apparent mainly at the eighth and twelfth inches from the surface but was slightly evident also down to 3 feet.

The results from plot 609 show that at the 4 inch depth the count obtained was greater than that of plots 601 and 602 but less than that of plots 604 and 607. At 8 inches however, the numbers present were not ony greater than those present in plots 601 and 602 but also greater than those in plot 604 but less than those in plot 607. At the lower depths the counts

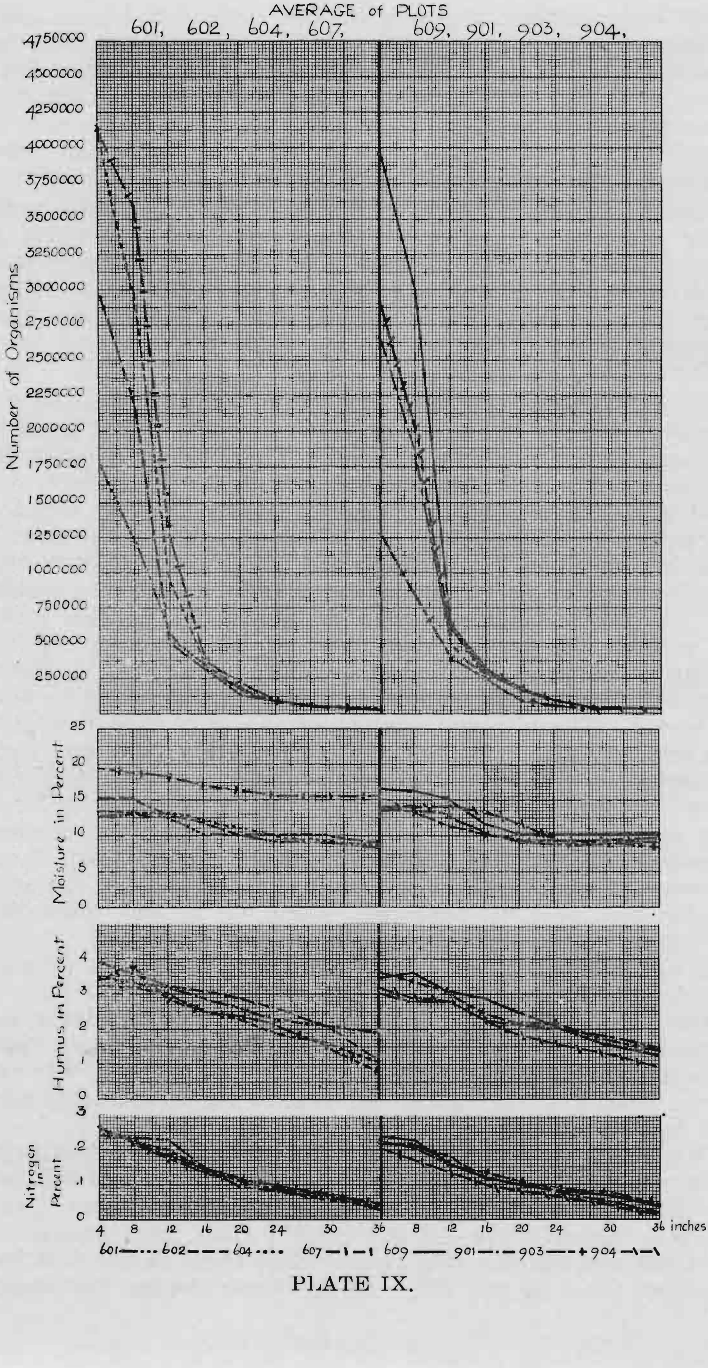
AVERAGE of PLOTS
601, 602, 604, 607, 609, 901, 903, 904,
Number of Organisms
4750000
4500000
4250000
4000000
3750000
3500000
3250000
3000000
2750000
2500000
2250000
2000000
1750000
1500000
1250000
1000000
750000
500000
250000
Moisture in Percent
25
20
15
10
5
0
Humus in Percent
4
3
2
1
0
Nitrogen in Percent
3
.2
.1
0
4 8 12 16 20 24 30 36 8 12 16 20 24 30 36 inches
601 602 604 607 609 901 903 904

PLATE IX.

were very similar to those obtained on the other plots. The moisture content of the soil from this plot was very much the same as that of the other soils; the humus content was not very different from that of the other soils and the nitrogen content was practically the same. Hence the difference observed in bacterial counts was evidently due to some other factor not examined. Again in this plot we find a greater humus content at 8 inches from the surface than at 4, 3.63% against 3.46%, and while at the four inch depth there were fewer bacteria in this plot than in 604, at the 8 inch depth the reverse was the case. There is some evidence from these last mentioned results of the effects of humus content on the numbers of bacteria in the soil.

The effect of turning under cowpeas for a green manure in a regular two-year rotation, therefore, seemed to be to increase the bacterial content of the soil over that of the soil under a regular two-year rotation. At the 8 inch depth the cowpeas increased slightly the number of organisms beyond the number present in the three-year rotation plot but all the other depths the counts obtained from the three-year rotation plot were greater showing the effect on bacteria by growing a crop of clover in the regular way to be greater than turning under a crop of cowpeas.

The averages from plot 901 under a two-year rotation with rye turned under for a green manure show that at the fourth and eighth inch depths the counts obtained were greater than those obtained at similar depths in the continuous corn plot, but less than those found in any of the other plots. Below the twelfth inch the numbers were very closely in agreement with those of the other plots, particularly 601. The moisture conditions in this plot were very similar to that of the other plots and the humus and nitrogen contents were practically the same and hence their effects were not noticeable. The effect therefore, of turning under a crop of rye in a two-year rotation seemed to be to depress the bacterial content of the soil below that of the soil under the regular two-year rotation but not below that of the continuous corn plot.

As has been mentioned plots 903 and 904 were in clover and can therefore hardly be compared with the others except in a general way. In plot 903, under continuous clover, at 4 8, 12 and 16 inches the numbers were smaller than those obtained in the continuous corn plot. At the lower depths the numbers were about the same as those in the other plots.

The moisture, humus, and nitrogen contents of the soil from this plot were about the same as that of the other plots, and the differences were therefore due largely to the differences in preparation for and treatment of the crop grown and possibly also to other unexamined factors. At any rate it is evident that the continuous clover plot contained fewer organisms than the continuous corn plot or any of the plots under the two or three-year rotations, the differences being very pronounced down to 2 feet but inconsiderable below that point.

In plot 904, under a four-year rotation, there were more bacteria at the fourth, eighth, twelfth and sixteenth inch depths than at similar depths in the continuously cropped plots or in the two-year rotation plot with rye turned under. At lower depths the differences were very slight. The moisture, humus, and nitrogen content in this plot were very much the same as in the other plots and here again the differences observed were due to the crop grown or to some other unrecorded factor. The effect of the previous cropping was apparent here, notwithstanding the fact that the crop of clover on the plot during the experiment reduced the number of organisms below that found in the other plots where corn was grown.

The larger increase over the continuous clover plot was also quite evident.

Considering these results as a whole, then, we find that the effects of different rotations and methods of cropping on the numbers of bacteria in the soil were clearly shown by the results obtained. Rotation of crops increased the number of organisms in every case beyond continuous cropping, the three-year rotation giving a larger increase than the two-year rotation or the two- year rotation in which clover or cowpeas were turned under. The two-year rotation with rye turned under showed fewer numbers than the regular two-year rotation. In every case the differences in bacterial content of the same layer of soil in different plots were greatest within the first foot while below that point the variations were not very great, although some evidence of the effect of rotation and methods of cropping was given even at the third foot. In the case of the soils where green manures were employed and where clover was grown in rotation, the differences were still large at the twelfth inch, while in the other plots the variations were not large below the eighth inch.

Other work which has been carried on by the author[1] with these same plots has shown that not only was the total number of organisms in the soil increased by the rotation of crops, but that the ammonifying, nitrifying, and nitrogen-fixing powers of the soil, as tested by the beaker method, were also increased, and that, furthermore, the crop produced from the soil was greater where rotation was practised, the corn crop from the three-year rotation plot being much larger than from the continuous corn plot, or from the two-year rotation plot, or from the plots under two-year rotation where clover, cowpeas, or rye were turned under for a green manure; and that finally turning

(1) Research Bull. No. 6, Iowa Expt. Sta.

under the crop of rye in the two-year rotation plot decreased the yield below that of the regular two-year rotation plot. This work therefore, confirms previous investigations regarding the effect of different rotations on the number of bacteria in the soil.

While in some instances there seemed to be some effect of moisture, nitrogen, and humus content on the numbers of organisms in these soils, in most cases the effects were nonapparent; that is, although the presence of more moisture and food material might be expected to increase the numbers of bacteria in the soil, such was not always the case. Some other factor evidently exerted more influence than any of these. We have found that neither the humus content nor the nitrogen content of the soil governed the number of organisms. While it is possible that the other mineral plant food constituents may have had some influence, it is hardly likely that there would be enough difference in the content of the different plots in potassium and phosphorus to explain the difference in bacterial content. Similarly the difference in reaction would probably be too slight to cause the variations which were observed.

We must turn, therefore, to the physical differences and as the moisture content of the soil was shown to be insufficiently variable to explain the differences, we must consider the question of aeration. These results suggest that possibly the difference in aeration in the different plots influenced the number of bacteria more than any other factors, or at least that it had a predominating effect over the effects of the other factors. No tests were made of the aeration in the plots at the different depths, owing to the lack of any satisfactory methods, but it seems probable from the results obtained that some effects would have been noted.

There is one other explanation of the results which seems probable and may be suggestd here. In a previous work already cited,[1] the writer called attention to the fact that possibly the cause of the depression on the bacterial flora of soils brought about by continuous cropping might be that the so-called toxic substances, examples of which have been isolated by the Bureau of Soils, are produced in the growth of plants and when rotations are not practiced they accumulate until their effect is deleterious to crop production. Furthermore, it was also suggested that when crops are rotated, the beneficial effect might be due largely to the neutralizing action or destroying action which the substances excreted from one crop might have on those substances left by another crop. If such toxic substances are produced in the growth of plants, then it is certain that they have some influence on bacterial life.

(1) Research Bull. No. 6, Iowa Expt. Sta.

The results of this work show that the effects of different rotations may be very noticeable on the numbers of bacteria and that none of the differences in moisture conditions, in humus content, and in nitrogen content, was sufficient to explain the effects. Further work is being planned to determine whether aeration may explain the differences, and although it seems hardly likely that there is enough difference in the aeration of plots arranged as they were in this series to explain the bacterial differences, such may be the case. If this is not so, then an examination of the toxic properties of the soil will be made and the isolation of substances produced in the growth of plants which are harmful to the succeeding crop of the same plant, will be attempted. From a study of the effects of such substances on the bacterial flora of the soil, we should be able to reach a conclusion as to whether or not the effects of continuous cropping and rotating on the bacteria in the soil should be attributed to the toxic substances.

CONCLUSIONS.

The conclusions which may be drawn from this work are:

1. There was a more or less regular decrease in numbers of organisms in all the plots from four inches down to three feet.
2. The greatest number of bacteria in all the plots was found at four inches from the surface.
3. The greatest decrease in numbers between any two zones sampled, occurred between the eighth and twelfth inches.
4. In every case the rotation of crops increased the number of organisms beyond continuous cropping.
5. The soil under the three-year rotation gave larger numbers than that under the two-year rotation, and at four inches from the surface, greater numbers than those soils under the two-year rotation with clover or cowpeas turned under, but at eight inches smaller numbers than the soils from these latter plots.
6. The number of organisms in the plot under the two-year rotation with rye turned under, was less than that in the regular two-year rotation plot.
7. The plot in continuous clover showed fewer organisms than that in continuous corn, due largely probably to the difference in treatment for the crop, as the greatest variation appeared near the surface and little difference was found below twelve inches.
8. The plot under the four-year rotation showed greater numbers than the continuous cropped plots or the two-year rotation plot with rye turned under, but fewer numbers than the two or three-year rotation plots or the two-year rotation plot with clover or cowpeas turned under. The difference in crop grown here was evidently largely responsible for the results.

9. The humus content of the soil in all the plots except two decreased more or less regularly from the surface down to three feet. In the plots under the two-year rotation with clover or cowpeas turned under, there was more humus at eight than at four inches from the surface.

10. In some instances there seemed to be some relation between humus content and numbers of organisms, but in most cases there was evidently none, and in fact sometimes high humus content and low numbers were coincident.

11. There was a more or less gradual decrease in nitrogen content in the soils from the surface down to three feet.

12. While in some cases, some effect of nitrogen content of the soil on the numbers of organisms was observed, such an effect was mostly non-apparent, and although it might be expected that the presence of more nitrogenous food should increase the number of bacteria, such was not the case here, and some other factor seems to have been of more importance.

13. The number of bacteria in the soils examined was shown not to be governed predominately by the nitrogen or humus content, and the moisture conditions were not sufficiently different to explain the variations in numbers.

14. It is suggested that aeration may be the important factor or that possibly the effect of toxic substances produced in the growth of plants may be the cause of the variations in the bacterial content of the different plots.

SERIES II.

The soil used in this series was obtained from an experimental orchard located near Council Bluffs on the light loess soil known as Missouri Loess. The soil was sampled and the plates made in the same way as has already been described in series I. Samples were taken every 4 inches down to 2 feet, and then every 6 inches down to 15 feet. Duplicate samples were drawn to a depth of 10 feet but below that only a single lot of samples was drawn.

The results of the quantitative determinations may be found in table V. and the moisture determinations in the samples are in table VI. The samples which were examined in this series were drawn June 26, 1911. It will be noted on examining the moisture table that the moisture conditions were quite uniform, and below the thirtieth inch there was very little further decrease down to the fifteenth foot where 14.00% was found.

We find here that the greatest decrease in numbers between any two depths occurred between the eighth and twelfth inches. It will be remembered that in series I, the greatest decrease occurred at that point. Below that rather a regular decrease occurred down to the fourth foot, when a large decrease occurred, and then a more or less gradual drop took place down to the fifteenth foot, where 244 bacteria per gram of air-dry soil were found. Previous work already cited has shown that bacteria were absent below 3 meters depth in the soil examined, but that work was evidently carried on with a much heavier soil, for here there were still over 200 organisms per gram of soil at a depth of 15 feet. Of course the Missouri loess soil is very light, very open, and the aeration is much greater than in most soils, and in this series the moisture conditions were rather high at the lower depths.

Comparing the results with those secured in series I, we find that at 3 feet, which was the greatest depth of sampling in that case, only about 30,000 bacteria were present per gram of soil, while here at 3 feet 218,000 organisms were present,—a very much larger number. Evidently the aeration conditions here were of great importance for the humus content of the soil was not nearly so great as in the Wisconsin drift soil, and the moisture conditions were not very different, so that the differ-

TABLE V. SERIES II.

Lab. No.	Depth of Sampling	Bacteria Per Gram of Air-dry Soil I.	II.	Average
I.	4 in.	4,424,000	4,404,000	4,414,000
II.	8 in.	2,266,000	2,256,000	2,261,000
III.	12 in.	836,000	809,000	822 500
IV.	16 in.	554,000	589,000	571,500
V.	20 in.	333,000	342,000	337,500
VI.	24 in.	284,000	278,000	281,000
VII.	30 in.	235,000	245,000	240,000
VIII.	36 in.	217,000	220,000	218,500
IX.	42 in.	203,000	203,000	203,000
X.	48 in.	156,000	147,000	151,500
XI.	54 in.	67,000	58,000	62,500
XII.	60 in.	45,000	44,000	44,500
XIII.	66 in.	31,800	30,800	31,300
XIV.	72 in.	25,300	25,700	25,500
XV.	78 in.	20,100	20,400	20,250
XVI.	84 in.	15,000	14,900	14.950
XVII.	90 in.	12,500	11,900	12,200
XVIII.	96 in.	9,400	10,000	9,700
XIX.	102 in.	7,800	8,200	8,000
XX.	108 in.	6,500	6,800	6.650
XXI.	114 in.	5,500	5,500	5,500
XXII.	120 in.	3,900	3,400	3,650
XXIII.	126 in.	3,200		3,200
XXIV.	132 in.	2,170		2,170
XXV.	138 in.	1,700		1,700
XXVI.	144 in.	1,310		1,310
XXVII.	150 in.	940		940
XXVIII.	156 in.	600		600
XXIX.	162 in.	347		347
XXX.	168 in.	311		311
XXXI.	174 in.	276		276
XXXII.	180 in.	244		244

TABLE VI.

MOISTURE IN SERIES II.

Lab. No.	Depth of Sampling	Percent Water I.	Percent Water II.
I.	4 in.	17.50	17.00
II.	8 in.	17.50	14.00
III.	12 in.	17.50	16.00
IV.	16 in.	17.00	15.50
V.	20 in.	16.00	15.50
VI.	24 in.	15.50	15.00
VII.	30 in.	15.00	14.00
VIII.	36 in.	12.50	13.00
IX.	42 in.	11.50	12.50
X.	48 in.	10.50	12.50
XI.	54 in.	10.50	12.50
XII.	60 in.	10.50	12.50
XIII.	66 in.	12.50	12.50
XIV.	72 in.	12.50	12.50
XV.	78 in.	12.00	12.00
XVI.	84 in.	12.00	12.00
XVII.	90 in.	12.00	12.50
XVIII.	96 in.	12.50	12.50
XIX.	102 in.	13.00	12.50
XX.	108 in.	13.00	13.00
XXI.	114 in.	13.00	13.00
XXII.	120 in...	13.00	13.00
XXIII.	126 in.	12.00	
XXIV.	132 in.	11.75	
XXV.	138 in.	12.50	
XXVI.	144 in.	12.75	
XXVII.	150 in.	13.00	
XXVIII.	156 in.	12.50	
XXIX.	162 in.	14.25	
XXX.	168 in.	14.00	
XXXI.	174 in.	13.50	
XXXII.	180 in.	14.00	

ence in mechanical composition and the consequent variations in the aeration were the governing factors in the growth of the soil organisms.

SERIES III.

The soils examined in this series were also taken from the experimental orchard at Council Bluffs, but were obtained October 9, 1911, after the long continuance of a severe drought, and when the moisture content of the soil was consequently very much lower and the number of organisms was very much smaller. The results of the quantitative determinations may be found in table VII, which gives also the moisture determinations. Samples were taken as before, but to a depth of only 5 feet, where 12,900 organisms were found against 44,500 at 5 feet at the previous date. The moisture content of the

TABLE VII. SERIES III.

Lab. No.	Depth of Sampling	Moisture in Samples Percent	Bacteria Per Gram of Air-dry Soil
I.	4 in.	15.50	2,500,000
II.	8 in.	10.50	1,960,000
III.	12 in.	8.00	852,000
IV.	16 in.	5.50	507,000
V.	20 in.	4.00	364,000
VI.	24 in.	4.00	212,000
VII.	30 in.	4.00	114,000
VIII.	36 in.	4.00	81.000
IX.	42 in.	4.00	47,700
X.	48 in.	4.00	40,000
XI.	54 in.	4.00	20,400
XII.	60 in.	4.00	12,900

soil from 20 inches down to 5 feet was uniformly 4.00%, and this compared with the 12.14% in the soil at the June sampling, undoubtedly largely explains the lower numbers. At 4 inches from the surface we find only 2,500,000 bacteria per gram of soil against 4,414,000 in June, but the greatest decrease between any two samples occurred in this series also between the eighth and twelfth inches. Comparing the results from this series with those of series I, we find that the number of bacteria in this soil at three feet was 81,000, while in series I, in the Wisconsin drift, it was almost 30,000, so that notwithstanding the very much lower moisture conditions in the loess soil, the number of organisms at three feet and in fact at every depth below the surface was much greater. Thus the results of series II were amply confirmed in series III, and showed that the mechanical composition of the soil and the consequently greater aeration was undoubtedly largely responsible for the greater numbers in the loess soil over those in the drift soil.

SERIES IV.

The samples examined in this series were taken at Humeston, in southern Iowa, on the Southern Iowa Loess. These samples were taken in duplicate in the usual way, and the results of the quantitative determinations are found in table VIII, while table IX gives the moisture determinations.

Looking over the moisture content of the soils, we find that there was less moisture at the four-inch depth than at any other point, and that while a gradually decreasing amount of water was found in the soil down to 5 feet, even at that depth there was still 15.50% present. The number of organisms decreased from the surface to 5 feet but the largest decrease here betwen any two samples occurred between the six-

TABLE VIII. SERIES IV.

Lab. No.	Depth of Sampling	Bacteria Per Gram of Air-dry Soil I.	II.	Average
I.	4 in.	5,190,000	5,480,000	5,335,000
II.	8 in	4,670,000	4,760,000	4,715,000
III.	12 in.	3,680,000	3,830,000	3,755,000
IV.	16 in.	2,500,000	2,870,000	2,685,000
V.	20 in.	695,000	725,000	710,000
VI.	24 in.	335,000	375,000	355,000
VII.	30 in.	185,000	212,000	198,500
VIII.	36 in.	118,000	128,000	123,000
IX.	42 in.	56,600	59,100	57,850
X.	48 in.	44,900	45,400	45,150
XI.	54 in.	26,000	26,500	26,250
XII.	60 in.	19,400	21,300	20,350

TABLE IX.

MOISTURE IN SERIES IV.

Lab. No.	Depth of Sampling	Percent Water I.	Percent Water II.
I.	4 in.	11.50	12.50
II.	8 in.	16.50	16.00
III.	12 in.	17.50	16.50
IV.	16 in.	20.00	20.00
V.	20 in.	20.00	20.00
VI.	24 in.	20.00	20.00
VII.	30 in.	19.00	19.00
VIII.	36 in.	17.00	16.00
IX.	42 in.	16.00	15.50
X.	48 in.	15.50	15.50
XI.	54 in.	15.50	15.50
XII.	60 in.	15.50	15.50

teenth and twentieth inches. These results we find agreed more closely with the results obtained on the Missouri loess in series II and III than with those obtained on the Wisconsin drift in series I. Thus, at 3 feet we find 123,000 bacteria per-gram of soil with a moisture content of 16.00%; in the Missouri loess, there were 218,500 bacteria with a moisture content of 14.00% and 81,000 with a moisture content of only 4.00% while in the Wisconsin drift there were only about 30,000 organisms with a moisture content of about 8.00%. Here again the number of organisms seemed to be governed very largely by the mechanical composition of the soil, and the consequent difference in aeration. The more open the soil the greater the number of bacteria, the aeration showing more influence than greater food supply.

SERIES V.

Series V consisted of an examination of samples of soil from another type of Wisconsin drift soil taken from a clover field in the college experimental area. This soil was much lighter than the Marshall loam of series I, and contained rather a large amount of sand and would be classed by the Bureau of Soils as Marshall Sandy loam. The samples were taken from the field in the usual way, and the results of the determinations are given in tables X and XI. There was considerable variation in the moisture content of the two samples, but the differences in the counts were not very great, and the average results will be considered. There was a very gradual decrease in organisms from the surface down to five feet, the greatest drop occurring here between the fourth and eighth inches. The numbers at the surface were less than those in the loess soils, and the numbers at three feet were less, but at this latter depth the numbers were much larger than those in the Marshall loam. The moisture conditions were not very different from those in series I, so that here again we may conclude that the mechanical condition of the soil and better aeration brought about the much larger numbers on the sandy loam.

TABLE X. SERIES V.

Lab. No.	Depth of Sampling	Bacteria Per Gram of Air-dry Soil I.	II.	Average
I.	4 in.	3,093,000	3,139,000	3,116,000
II.	8 in.	2,044,000	2,186,000	2,115,000
III.	12 in.	1,283,000	1,133,000	1,208,000
IV.	16 in.	652,000	630,000	641,000
V.	20 in.	484,000	432,000	458,000
VI.	24 in.	189,000	167,000	178,000
VII.	30 in.	124,000	120,000	122,000
VIII.	36 in.	96,800	89,800	93,300
IX.	42 in.	64,900	65,100	65,000
X.	48 in.	54,400	49,400	51,900
XI.	54 in.	46,000	42,900	44,450
XII.	60 in.	33,900	25,300	29,600

TABLE XI.
MOISTURE IN SERIES V.

Lab. No.	Depth of Sampling	Percent Water I.	Percent Water II.
I.	4 in.	19.50	14.00
II.	8 in.	20.00	14.00
III.	12 in.	16.50	10.00
IV.	16 in.	15.00	8.00
V.	20 in.	15.00	7.50
VI.	24 in.	15.00	7.00
VII.	30 in.	15.00	7.00
VIII.	36 in.	15.00	11.00
IX.	42 in.	14.50	11.00
X.	48 in.	14.50	11.00
XI.	54 in.	14.50	11.00
XII.	60 in.	14.50	11.00

SERIES VI.

Samples were taken in the usual way from a typical wood soil, and the results are given in tables XII and XIII. The moisture conditions were very uniform and the average results very satisfactory. The greatest decrease between any two layers occurred between the fourth and eighth inches and beyond that there was rather a regular decrease down to five feet. At every zone there were much smaller numbers than in either the loess soils or the sandy loam, but that was not the case with all the loam plots. In some of these there were fewer organisms at tweleve inches than in the woods soils, and fewer also at twenty inches, although the difference here was very slight and might be partly, at least, accounted for by difference in moisture conditions. The effect of cultivation was, therefore, very clearly shown here. Of course this effect was probably not entirely due to differences

TABLE XII. SERIES VI.

Lab. No.	Depth of Sampling	Bacteria Per Gram of Air-dry Soil I.	II.	Average
I.	4 in.	2,045,000	2,010,000	2,027,500
II.	8 in.	1,340,000	1,315,000	1,327,500
III.	12 in.	910,000	875,000	892,500
IV.	16 in.	324,000	214,000	264.000
V.	20 in.	172,000	169,000	170,500
VI.	24 in.	75,500	74,500	75,000
VII.	30 in.	22,000	21,500	21,750
VIII.	36 in.	14,000	15,500	14,750
IX.	42 in.	8,600	8,900	8,750
X.	48 in.	6,480	6,820	6,650
XI.	54 in.	5,000	5,200	5,100
XII.	60 in.	3,960	4,340	4,150

TABLE XIII.
MOISTURE IN SERIES VI.

Lab. No.	Depth of Sampling	Percent Water I.	Percent Water II.
I.	4 in.	14.50	15.50
II.	8 in.	15.00	16.50
III.	12 in.	12.50	12.00
IV.	16 in.	10.50	12.00
V.	20 in.	10.50	12.00
VI.	24 in.	10.50	11.50
VII.	30 in.	10.00	11.50
VIII.	36 in.	10.00	11.50
IX.	42 in.	11.50	12.00
X.	48 in.	12.50	12.50
XI.	54 in.	14.00	12.50
XII.	60 in.	13.00	12.50

in mechanical composition or in aeration, but also due to the accumulation of acid organic matter in woodland soils, which as is well known, limits bacterial activity and reduces the number of organisms. These results, therefore, confirm formmer observations that there are fewer bacteria in woodland soils than in cultivated soils.

SERIES VII.

In series VII, samples were taken as usual from an experimental field located on the Marshall loam, but underlaid by a much stiffer subsoil than was the case with the soils used in series I. Consequently we find from the results in tables XIV and XV that while the moisture conditions were very much the same as in series I, the number of organisms was greater down to two feet but below that it was less or about the same, showing that there may be considerable variation

TABLE XIV. SERIES VII.

Lab. No.	Depth of Sampling	Bacteria Per Gram of Air-dry Soil I.	II.	Average
I.	4 in.	4,310,000	4,180,000	4,245,000
II.	8 in.	4,150,000	3,800,000	3,975,000
III.	12 in.	2,820,000	2,430,000	2,625,000
IV.	16 in.	530,000	544,000	537,000
V.	20 in.	266,000	264,000	265,000
VI.	24 in.	116,000	109,000	112,500
VII.	30 in.	47,900	45,800	46,850
VIII.	36 in.	28,500	26,600	27,550
IX.	42 in.	20,000	18,900	19,450
X.	48 in.	14,600	15,000	14,800
XI.	54 in.	10,400	9,900	10,150
XII.	60 in.	8,780	8,700	8,740

TABLE XV.

MOISTURE IN SERIES VII.

Lab. No.	Depth of Sampling	Percent Water I.	Percent Water II.
I.	4 in.	16.50	14.50
II.	8 in.	21.50	19.00
III.	12 in.	22.00	16.50
IV.	16 in.	19.00	15.50
V.	20 in.	19.00	15.00
VI.	24 in.	19.00	14.50
VII.	30 in.	19.00	13.00
VIII.	36 in.	18.00	12.50
IX.	42 in.	17.50	15.50
X.	48 in.	17.00	16.00
XI.	54 in.	16.50	17.50
XII.	60 in.	16.00	17.50

in the bacterial content of the same type of soil under slightly different mechanical and subsoil conditions.

CONCLUSIONS.

The results of these studies of bacteria at different depth show that:

1. In every soil examined the greatest number of organisms occurred at four inches from the surface.

2. Bacteria were found in considerable numbers at much lower depths in the loess soil than in the drift soil.

3. There was a more or less gradual decrease in numbers down to 5 feet, and in one case down to 15 feet. In no case were the sudden increases observed which other writers have described, even though in some cases increases in moisture conditions were found.

4. There is indication from the results that aeration conditions may have been of more influence on the numbers than the moisture content, but it is impossible to state definitely what was the governing factor.

5. The most rapid decrease in organisms occurred within the first 12 inches, and was sometimes limited to the first 8 inches, showing that although the moisture conditions might be almost identical, the numbers diminished very rapidly with increasing distance from the surface.

The results of the entire work are not accepted as being representative of all soils of the same types, for it is clearly recognized that slight differences in mechanical composition, in topography, in climatic and weather conditions, in cropping, etc., may bring about striking variations.

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